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FINAL REPORT

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PREVENTION OF THE COLONIZATION OF POULTRY BY CAMPYLOBACTER JEJUNI: MODEL DEVELOPMENT AND VACCINATION STUDIES

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Prevention of colonization of poultry by *C. jejuni*: Model development and vaccination studies

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SUMMARY

Campylobacter jejuni is responsible for more cases of gastroenteritis in the developed world than Salmonella or any other food borne pathogen. This project had two objectives: 1. To develop suitable animal models and 2. To prove that vaccination is an effective method for the prevention of colonization of poultry by *C. jejuni.*

We have successfully competed the first objective. We have developed two types of models for the colonization of poultry by *C. jejuni.* In the standard model all birds are challenged with the bacterium. In horizontal transfer model 20 percent of the birds are challenged and then they are commingled with the unchallenged birds. These models work with birds from 1 day of age to 4 weeks.

We have studied the effect of vaccination of hens on the ability of *C. jejuni* to colonize their progeny. We have also studied the effect of vaccination on the ability of *C. jejuni* to colonize the hens as well. In neither case did vaccination decrease the colonization observed. This failure may be due to the antigen used. Further studies are underway to determine what proteins are important in the colonization of poultry by *C. jejuni.*

TECHNICAL REPORT

Background

Campylobacter jejuni is responsible for more cases of gastroenteritis in the developed world than Salmonella or any other food borne pathogen. Data from Health Canada Notifiable Diseases On-Line suggest that Campylobacter is responsible for more than 50% of foodborne and waterborne bacterial infections in Canada in 1999. One significant risk factor for the transmission of Campylobacter to humans is the consumption and handling of uncooked poultry. Although *C. jejuni* does not cause disease in poultry it is believed to be a major source of human infection by Campylobacter. A significant proportion of chicken meat is contaminated with Campylobacter at the time of retail sale. Workers in the poultry industry are also believed to be at greater risk than the general population. Most poultry flocks are not contaminated before two weeks of age but by time of slaughter up to 100% of the flocks are colonized by *C. jejuni*. The organisms are ubiquitous in the environment and this makes exclusion by use of biosecurity generally unfeasible; however some flocks remain free of Campylobacter. New and effective control procedures, such as vaccination of poultry can be designed after we have better understanding of the mechanism of colonization of poultry by *C. jejuni.* This will lead to increased safety for workers in the food industry and increased food safety in Canada.

Project Objectives

The Long Term Objective: **To develop a vaccination strategy that will be effective in the prevention of colonization of poultry by *C. jejuni.***

The Specific goals of this project are:

1. To develop suitable models for colonization of poultry by *C. jejuni.*
2. To prove that vaccination is an effective method for the prevention of colonization of poultry by *C. jejuni.*

This work will provide information that will form the basis for the development of a vaccine for the prevention of colonization of poultry by *C. jejuni.* We believe this will be an effective strategy for the control of this important food-borne pathogen in poultry and will lead to a reduction in the cases of *Campylobacter* enteritis that occur in developed countries.

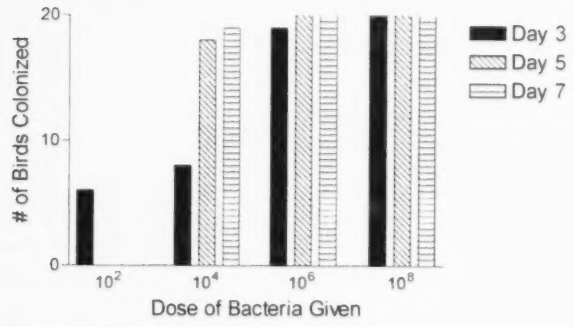
Research Results

1. Model Development

i. Development of one- day old chick model.

We have developed a model for the colonization of day old chicks. Birds, at one day of age were given an oral challenge of *C. jejuni* in a 0.5 mL volume (20 birds per group). Doses of *C. jejuni*, between 10^2 and 10^8 bacteria, were given. Colonization of the birds was monitored over a week. Cloacal swabs were taken and cultured for *C. jejuni* on selective media.

Fig. 1. Dose response of colonization by C. jejuni NCTC11168.



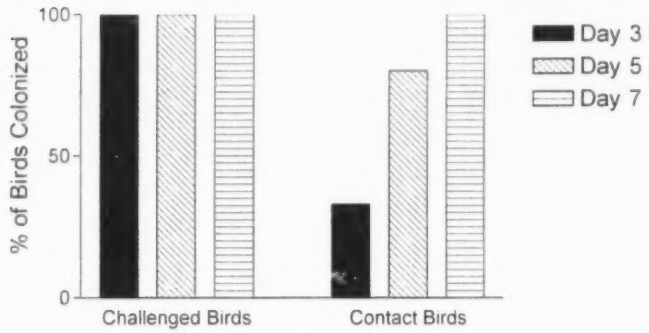
A dose of 10^6 *C. jejuni* per bird was sufficient to colonize over 90% of the birds. When 10^4 *C. jejuni* were given not all birds were colonized on day 3. By day 7 over 90% of the birds were colonized. This suggested that it would be possible to colonize birds by horizontal transfer of the bacteria from on bird to another.

ii. Development of a horizontal transfer model in one-day old chick model.

The dose response data (Fig. 1) suggested that it would be possible to colonize birds by horizontal transfer of the bacteria from one bird to another. This would more closely mimic the situation in the field where it is believed that a few birds are infected and the infection spreads from bird to bird.

To test this hypothesis we challenged five birds with a dose of 10^8 colony-forming units of *C. jejuni*. These birds were housed with 20 birds that had not been challenged. Cloacal swabs were taken on Day3, 5, and 7 after challenge to monitor the colonization of the contact birds. One week after the birds were exposed to the challenged group all of the contact birds were colonized. We believe this will be an excellent model from our work.

Fig. 2. Colonization of day old chicks by horizontal transfer.



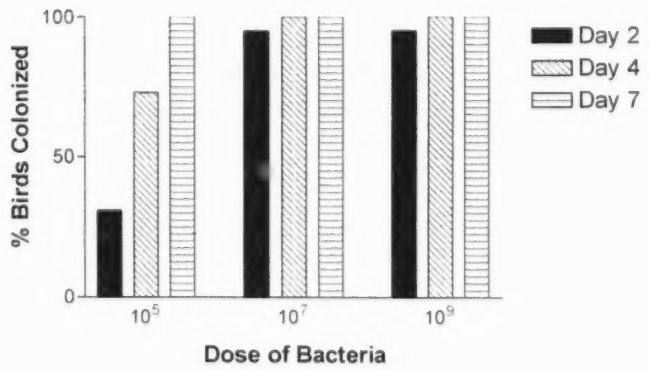
iii. Effect of type of chick used.

The initial experiments were done using Leghorn chicks. To confirm that exposure of broiler chicks to *C. jejuni* resulted in a similar colonization the experiment was repeated using broilers. No differences were observed between the types of birds used.

iv. Development of a model for the colonization of four-week old birds.

At four weeks of age birds were exposed orally with three doses of a standard strain of *C. jejuni*. Colonization was monitored for 7 days. Cloacal swabs were taken from all of the birds and cultured on selective media to detect Campylobacter.

Fig. 3. Colonization of four-week old birds by *C. jejuni*.

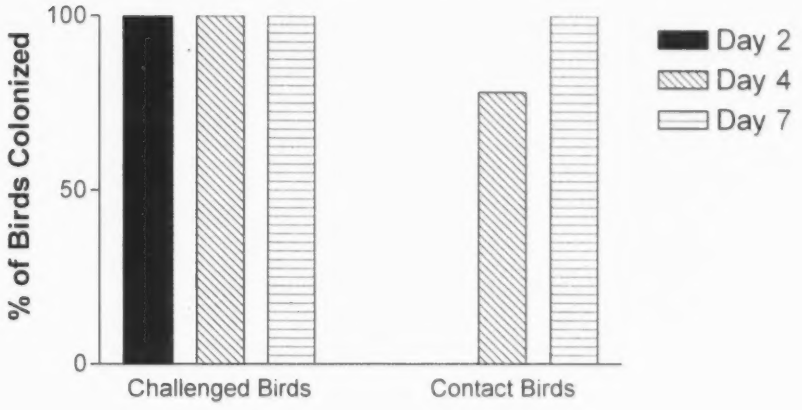


As few as 10^5 *C. jejuni* used as an oral challenge caused all of the birds to shed Campylobacter.

v. Development of a horizontal transfer model in four-week old birds.

A second experiment was carried out to determine if four-week-old birds could be colonized by horizontal transfer. Twenty percent of the birds were given 10^8 bacteria per bird. The remaining birds were place in contact with the challenged birds. All birds were monitored for colonization as described above.

Fig. 4. Colonization of four-week old birds by horizontal transfer.



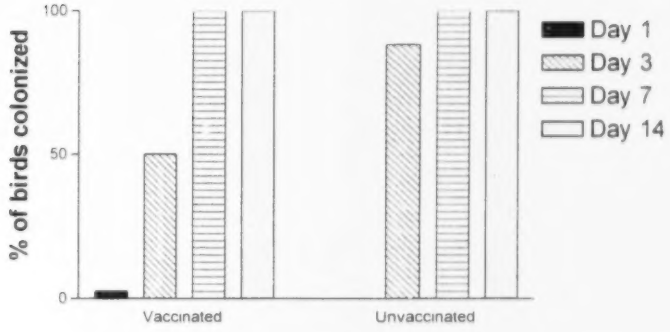
All of the challenged birds were colonized by day 2 and remained colonized throughout the experiment. By day 4 colonization could be detected in 75 percent of the contact birds and by day 7 all contact birds were colonized.

2. The Effect of Vaccination on the Colonization of Poultry by *C. jejuni*

i. The effect of immunizing hens on the colonization of progeny by *C. jejuni.*

Laying hens were immunized using killed *C. jejuni* cells in an adjuvant. The control birds receive adjuvant only. After three immunizations fertile eggs were collected from each group and hatched. Birds were divided into three groups of forty chicks. Two of the groups (1 & 2) were chicks from the control hens and the third group from the immunized hens were tagged and commingled. At day of age group one was challenged with 10^8 cfu's of *C. jejuni*. This dose of challenge resulted in the colonization of all birds in group one. The colonization of the remaining two groups was monitored over 14 days.

Fig. 5. The effect of vaccination of hens on the colonization of day old chicks by *C. jejuni*.



By 7 days of age chicks from vaccinated and unvaccinated hens were all colonized. They remained colonized until they were two weeks of age when the experiment was terminated.

ii. The effect of immunization on the colonization of hyperimmunized hens.

To determine if the hyperimmunized hens were colonized at a different rate than the control hens a horizontal transfer experiment was carried out. Twenty immunized and forty unimmunized hens were commingled. Half of the unimmunized birds were given 10^8 cfu's of *C. jejuni* orally. The birds were monitored by taking cloacal swabs on day 1,3,5 and 7 post challenge. No difference was observed in the colonization of either group of hens.

3. Conclusions and Future Directions

We have successfully developed models for the colonization of poultry by *C. jejuni*. These models can readily be adapted for use with other strains of *C. jejuni* with different colonization potential (data not shown). These models have already proven very useful in determining factors involved in the colonization of poultry by *C. jejuni*.

We have not been successful protecting either hens or their progeny from colonization with *C. jejuni* by vaccination. This may be due to the use of an inappropriate antigen or route of delivery. Further work to understand how *C. jejuni* and the avian host interact will allow us to make more appropriate decisions about the vaccination strategy.

PERSONNEL AND EQUIPMENT

No funds were requested for personnel or equipment.

PROJECT ACTIVITIES

Amoako, K., Gomis, S., Potter, A., and B. Allan. 2002. A model for the horizontal transfer of *Campylobacter jejuni* in poultry. Canadian Society of Microbiologists Annual Meeting Saskatoon, Saskatchewan, June 2002.

Allan, B. 2002 Poultry Infections by *Campylobacter jejuni* and their control. Poultry Service Industry Workshop. Banff , Alberta. October 2002 (Invited speaker).

Allan, B. 2002. Comparison of the ability of *C. jejuni* strains to colonize poultry. Presentation to the Institute for Biological Sciences, National Research Council. December 2002.

Carrillo, C. E. Taboada, J. Nash, P. Lanthier, J. Kelly, K. Amoaka, S. Gomis, P. Lau, R. Verhulp, J. Austin, B. Allan, and C. Szymanski. Genome-wide expression analyses of *Campylobacter jejuni* NCTC11168 reveals coordinate regulation of motility and virulence by *flhA*. For submission to J. Biological Chemistry.

ACKNOWLEDGEMENTS

Funding from ADF was acknowledged during the oral presentations and will be acknowledged in the paper that is in preparation for submission.